



Long-term changes in the structure of benthic communities: Revisiting a sampling transect in Crete after 24 years



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ABSTRACT

A bathymetric transect in the north coast of Crete first studied in 1989, was revisited 24 years later. Identical sampling design, season, techniques and protocols were followed in both studies in order to minimize bias in the long-term comparisons. This comprehensive macrofaunal dataset (4 stations, 2 sampling seasons, 7 replicates in each study) revealed changes in benthic diversity and community composition between the sampling periods. The recorded changes were higher at the stations located close to the coastal zone. In addition, while benthic communities showed lower total abundance during the recent sampling period, species abundances were more evenly distributed indicating that some species dominated the historical communities. In spite of these changes, the ecological status remained above the threshold values for good ecological status. The results indicated that changes in the benthic community seem to have been driven by local anthropogenic factors and natural variability rather than by large-scale factors such as nutrients influxes in the entire Mediterranean Basin.

1. Introduction

The Mediterranean Sea is an almost entirely enclosed sea, connected to other sea areas through the Strait of Gibraltar in the west and through the Suez Canal in the east. Economic and urban development is concentrated mainly in the coastal areas which are impacted by pressures affecting the marine ecosystem in terms of eutrophication, pollution, overfishing, climate change and the introduction of invasive species (Coll et al., 2012; Halpern et al., 2008; Karydis and Kitsiou, 2012; Lejeusne et al., 2010; Micheli et al., 2013; Powley et al., 2014). Despite the increasing trend of nutrient concentrations due to atmospheric and terrestrial influxes that has already been documented since 1998 (Béthoux et al., 1998), the eastern part of the Mediterranean remains ultra-oligotrophic due to water circulation pattern in the Mediterranean sea (Powley et al., 2014). In such nutrient-limited ecosystems, temporal changes are not exclusively the result of local effects but may follow large-scale changes superimposed onto the general circulation in the Mediterranean Sea (Katara et al., 2008).

Pelagic primary productivity in the Mediterranean was associated with important climatic patterns of the northern hemisphere and the water flow through the Strait of Gibraltar and Sicily (Katara et al., 2008; Powley et al., 2017, 2014). The North Atlantic Oscillation (NAO)

and the Mediterranean Sea oscillation through regulating changes in wind and precipitation regime, strongly affect chlorophyll a (Chl a) concentration and distribution in the Mediterranean (Katara et al., 2008). Moreover, atmospheric deposition of phosphorus in the eastern Mediterranean through Saharan dust depends on the polluted air masses from Europe, Africa, and the Middle East (Krom et al., 2004) and, as a result, it is controlled by climatic factors. This correlation in pelagic processes and climatic factors has been also extended to benthic communities where NAO induced resuspension events have changed macrofaunal abundance and biomass (Labrune et al., 2007). As a result, it is difficult to infer whether temporal changes in marine communities in such environments are due to human related activities, or whether they reflect natural variability in relation to decadal oscillatory atmospheric processes.

On the other hand, the assessment of the Ecological Quality Status of European marine waters associated with policy requirements, such as the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC), require a methodology that includes this natural variability. Specifically, the evaluation techniques have been generally based on some method of comparison with reference conditions in areas lacking human disturbances but characterized by natural variability (European Commission, 2003). In

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this context, the establishment of reference values on a local scale and the use of appropriate metrics that are not sensitive enough to fluctuate in natural variability, but still able to indicate human induced changes are of great importance (de Paz et al., 2008).

Marine benthic macrofauna has been regarded as a good indicator of environmental changes and consequently it is an important component in ecological quality status assessments (Borja et al., 2000; Dimitriou et al., 2012; Pearson and Rosenberg, 1978; Rosenberg et al., 2004; Simboula and Zenetos, 2002). Benthic communities are subject to many factors assumed to influence the coexistence of species such as food web interactions, including competition and predation, habitat specialization and environmental temporal heterogeneity (Gray and Elliott, 2009). Changes in community composition and species co-occurrence could be indicative of environmental changes (Heino and Grönroos, 2013).

One of the difficulties in correlating ecological shifts to driving factors is the relative absence of long-term time series (Clare et al., 2017). The establishment of reference values in benthic ecosystems is dependent upon the availability of long-term time series and historical data. While long-term time series monitoring benthic macrofauna composition are rare in the Mediterranean (Salen-Picard and Arlhac, 2002), comparison of current with historical data has been already used in many cases and was effective to infer temporal changes (Bonifácio et al., 2018; Grémare et al., 1998; Kress et al., 2004; Labrune et al., 2007; Romero-Ramirez et al., 2016).

In 1985, under the auspices of the NATO Science for Stability programme and the General Secretariat for Research and Technology a comprehensive programme (GR-FISHECO) was initiated by the Institute of Marine Biology of Crete in the Cretan Sea and the Southern Aegean. The aim of this project was to investigate the structure and dynamics of benthic communities of the shelf ecosystem. In the present paper, we compared data derived from the aforementioned project with recent data in order to identify the long-term temporal changes in the soft-bottom benthic community structure. Specifically, a transect line in the coastal area north of Crete (Eastern Mediterranean) revisited in 2013–14 and samples taken were compared to those taken during the period 1989–90.

The study area is an open bay that is difficult to accumulate wastes. It is under slight pressure mainly from discharges of domestic wastes and nutrients enrichment from agriculture (Simboula et al., 2016). The construction of the Heraklion municipality wastewater treatment plant in 22th April 1996 further reduced the organic enrichment by Heraklion city waste discharges removing annually $> 160,000$ kg of dry solids. As a result, the long-term comparison of the benthic communities in this area might reveal changes caused by natural variability rather than by human activity impacts. In this context, the objectives of the present paper were: (a) to assess the changes in macrobenthic communities and (b) to contribute in the assessment of the effectiveness of ecological quality status indices to remain unaffected from natural fluctuations. For this purpose, the following hypotheses were tested: (i) there is no change in macrofaunal diversity, (ii) there is no change in the overall community composition, (iii) there is no change in the set of dominant taxa identified in each sampling station, (iv) there is no change in species co-occurrence patterns and (v) there is no change in ecological status as it is revealed through different benthic indicators.

2. Materials and methods

2.1. Study site and sampling strategy

For the present study, we revisited a transect line located in Heraklion bay, Crete (south Aegean Sea) (Fig. 1). It included four sampling sites at 70, 100, 130 and 160 m depth, characterized by 90–95% silt and clay content. The continental shelf along the north coast of Crete forms a relative open bay in front of Heraklion city. Human pressure is slight and is related to sewage discharges and

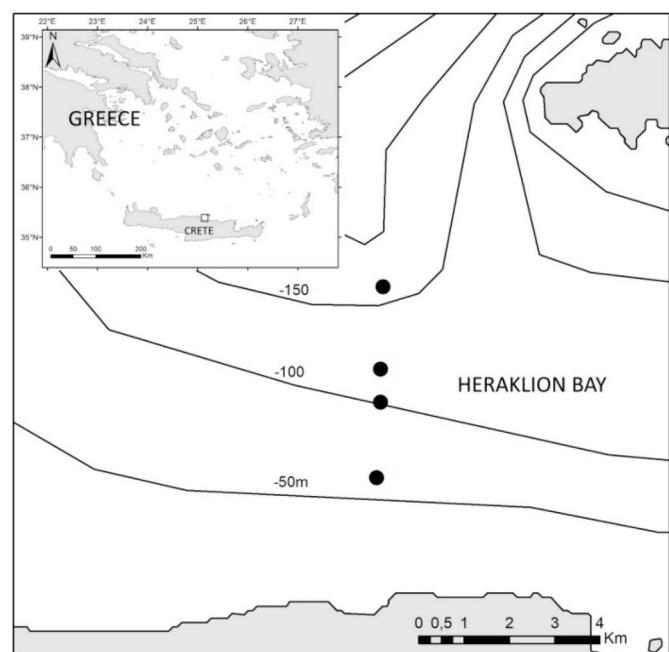


Fig. 1. Map of the sampling stations. Black circles indicate the four stations.

effluents form the adjacent city port (Simboula et al., 2016).

Sampling took place in November 2013 and May 2014. At each station, during each sampling occasion, 7 replicates were collected for macrofaunal analysis using a 0.1 m^2 Smith-McIntyre grab. Macrofaunal samples were sieved *in situ* over a 0.5 mm mesh, fixed with 5% buffered formalin, stained with Rose Bengal and stored for species identification. Additional triplicate cores (5 cm of diameter) were also collected for surface sediment analyses (0–2 cm layer), frozen and stored at -20°C . During the sampling, sediment temperature (T) and redox potential (Eh) were measured at the water-sediment interface by means of an electrode (METTLER TOLEDO; model PT4805-S7/120) (Wildish et al., 1999).

2.2. Laboratory analyses

Benthic macrofauna samples were sorted by hand and specimens were identified to the species or to the lowest possible taxonomic level and then counted. Sediment samples were analyzed for pigment and organic matter concentration. Chlorophyll a (Chl a) content was measured according to the method described by Yentsch and Menzel (1963) using a Turner fluorometer following extraction with 90% acetone. The loss on ignition method was used to determine the percentage of labile and refractory organic matter in sediments: organic material was determined as the weight loss of the dried sample after combustion for 16 h at 250°C for labile organic matter (LOM) and after following combustion for 16 h at 500°C for refractory organic matter (ROM) (Loh et al., 2008).

2.3. Data comparison and quality control

Macrofaunal data collected during the present sampling campaign were compared to the historical data obtained in 1989–90 during a previous research project in the sampling site (Karakassis et al., 1996; NATO Science for Stability Programme and FISHECO Project, 1992). Both sampling campaigns followed identical experimental designs and methodology in terms of sampling gear, techniques and sampling times. Nevertheless, quality control is an important process in order to avoid difficulties arising from typographic errors or misrepresentations in data. Changes in classification that occurred in the past 25 years and

synonymized species were handled by cross-checking, verifying and taxonomically updating all scientific names using the Taxon Match tool of the World Register of Marine Species (WoRMS) (WoRMS Editorial Board, 2017). To address the issue of differences in taxonomic resolution (not all individuals identified at species level) between the surveys, and also the bias caused during the identification procedure and different taxonomic expertise between samplings, all analyses performed on data aggregated at the genus level (Bonifácio et al., 2018; Bortolus, 2008; Labrune et al., 2007; Ranasinghe et al., 2003). In addition, individuals identified at a higher taxonomic level than species were pooled as Taxon sp.. Specifically, in both studies some individuals belonging to the taxonomic group of Sipuncula were not identified at species level. In such cases, species representative in both samplings were retained but unidentified individuals belonging to this taxon were pooled as Sipuncula sp. even if they had been identified at species level in one of the samplings.

2.4. Data analyses

Biodiversity and ecological quality status indices were calculated for all stations. Diversity indices included richness (number of taxa per sampling gear (S)), total abundance per sampling gear (N), Pielou's evenness (J'), Hurlbert's expected number of species (ES_{10}), log₂ Shannon diversity index (H') and Simpson diversity index ($1-\lambda$). Benthic quality indicators included M-AMBI (Muxika et al., 2007), BENTIX (Simboura and Zenetos, 2002) and BQI at family level (BQI-family) (Dimitriou et al., 2012). BQI-family was calculated in order to verify the M-AMBI and BENTIX results at a higher taxonomic level. Three-way ANOVA was performed to test the variability in these variables among the factors year, season and depth. To check the assumptions of approximately normal data and homogeneity of variance required for three-way ANOVA, Shapiro-Wilk test and Levene's test was performed. The more strict criterion of $\alpha = 0.01$ was used to reject null hypotheses when variances were heterogeneous (Underwood, 1996). When the three-way ANOVA indicated that there were significant differences within the dataset, Bonferroni test was used as a post hoc test between pairs of factors. The Pearson correlation was used to assess the relationship between the values of the aforesaid ecological indicators. To test for changes in environmental variables between 1989–90 and 2013–14, t-tests were used. The IBM SPSS Statistics 19 software was used for the analyses.

Relative frequency of occurrence for all species in all stations was calculated. Four classes of occurrence were defined according to Obst et al. (2017). The threshold for rare taxa was defined at 10% of the total locations and at 50% for common occurrence taxa.

Based on the total number of individuals and genera recorded for each sampling year, sample-based rarefaction and extrapolation curves were derived (Colwell et al., 2012). Rarefaction and extrapolation were calculated using the software EstimateS version 9 (Colwell, 2013) and the curves were plotted using individuals as an index of sampling effort.

Multivariate analysis of abundance data included non-metric multidimensional scaling (nMDS) using the Bray-Curtis similarity index and fourth-root transformation (to weight the contribution of common and rare species) (Clarke et al., 2014) in order to evaluate changes in community composition. Permutation multivariate analysis (PERMANOVA), using Bray-Curtis dissimilarity of fourth root transformed data, tested for differences in benthic community structure between the sampling years, seasons and depths. The SIMPER analysis (Clarke and Gorley, 2015) was used to identify the top ten genera that contributed mostly to dissimilarities in community composition among the sampling years. For the multivariate analyses, the Primer 7 statistical package was used (Clarke and Gorley, 2015). Functional traits for these genera related to feeding type and bioturbation were obtained from Polytraits database (Polytraits Team, 2018) and Queirós et al. (2013). Genera member-species share a large number of common biological and ecological characteristics such as feeding types (Fauchald and Jumars,

1979) and response to various types of stress (Dauvin et al., 2003) and as a result traits could be assigned to genera.

Relationships between the benthic community composition and the environmental factors were assessed using redundancy analysis (RDA), which is a linear method of direct ordination (Leps and Smilauer, 2003; ter Braak, 1994). RDA was performed using CANOCO 5 (ter Braak and Smilauer, 2012), with forward selection to identify the environmental variables that best explained the community composition. Environmental variables included in RDA were season, depth, sampling period, Eh, T and Chl a. The significance of the variables and the first ordination axis were determined using Monte Carlo permutation testing (499 permutations), implemented also in CANOCO 5.

In order to evaluate the community associations, the probabilistic model of species co-occurrence (Griffith et al., 2016; Veech, 2013) implemented in the R programme language (R Development Core Team 2017) into the “cooccur” package. This analysis measures co-occurrence in a straightforward way as the number of sampling stations where two taxa co-occur by comparing observed and expected co-occurrence patterns. The analysis was conducted using past (1989–90) and present (2013–14) macrofaunal communities data which results in different genera pair classes according to probability co-occurrence statistics (positive $P > 0.01$, negative $P < -0.01$ or random $-0.01 \leq P \leq 0.01$ association).

3. Results

3.1. Sediment characteristics

Environmental variables recorded in 1989–90 and 2013–14 are presented in Table S1 and Table S2 (See Supplementary materials section). Redox potential, Chl a and organic matter did not exhibit seasonal variability (between autumn and spring) at any depth. Redox potential ranged from 240 to 370 mV; Chl a from 0.01 to 0.56 µg/g, with the highest values recorded at the shallower station; and organic matter from 0.6% to 1.8%. Sediment temperature was approximately 17 °C in spring and 19 °C in winter. Significant differences between the two sampling periods were recorded for temperature (t -test; $t = -4.167$, $p < 0.001$) and redox potential (t -test; $t = 7.692$, $p < 0.001$).

3.2. Species diversity trends

A total of 4571 individuals belonging to 277 taxa were identified in 2013–14 and were compared to 10598 individuals belonging to 232 taxa from the historical dataset of 1989–90. Changes in the univariate indices among the factors year, season and depth and their statistical significance are shown in Fig. 2 and Table 1, respectively. Significant two-way interactions were recorded for total abundance and the pairwise comparisons separated the two sampling periods at each season and among all depths (Table S3). Specifically, total abundance was generally decreased between 1989–90 and 2013–14, except for the stations at 130 m and 160 m that shown a small increase in spring (Table 1 and Fig. 2). Three-way interaction was significant only for species richness, which indicated that the effect of the year*depth interaction was different among the seasons (Table 1). The pairwise test revealed that species richness was significantly decreased in winter at all depth stations, while in spring significant changes were recorded only at the two shallower stations (Table S4 and Fig. 2). Although, Shannon and Simpson diversity indices; and ES_{10} varied across different combinations of the factors depth and season, the factor year had a simple main effect on these variables (Table 1). As a result, it can be concluded that they were generally decreased between the two sampling periods (Fig. 2). On the opposite, Pielou's evenness did not significantly change between the years (Table 1 and Fig. 2).

Temporal changes in the relative frequency of species presence in all samples indicated decreasing occurrences and changes in community

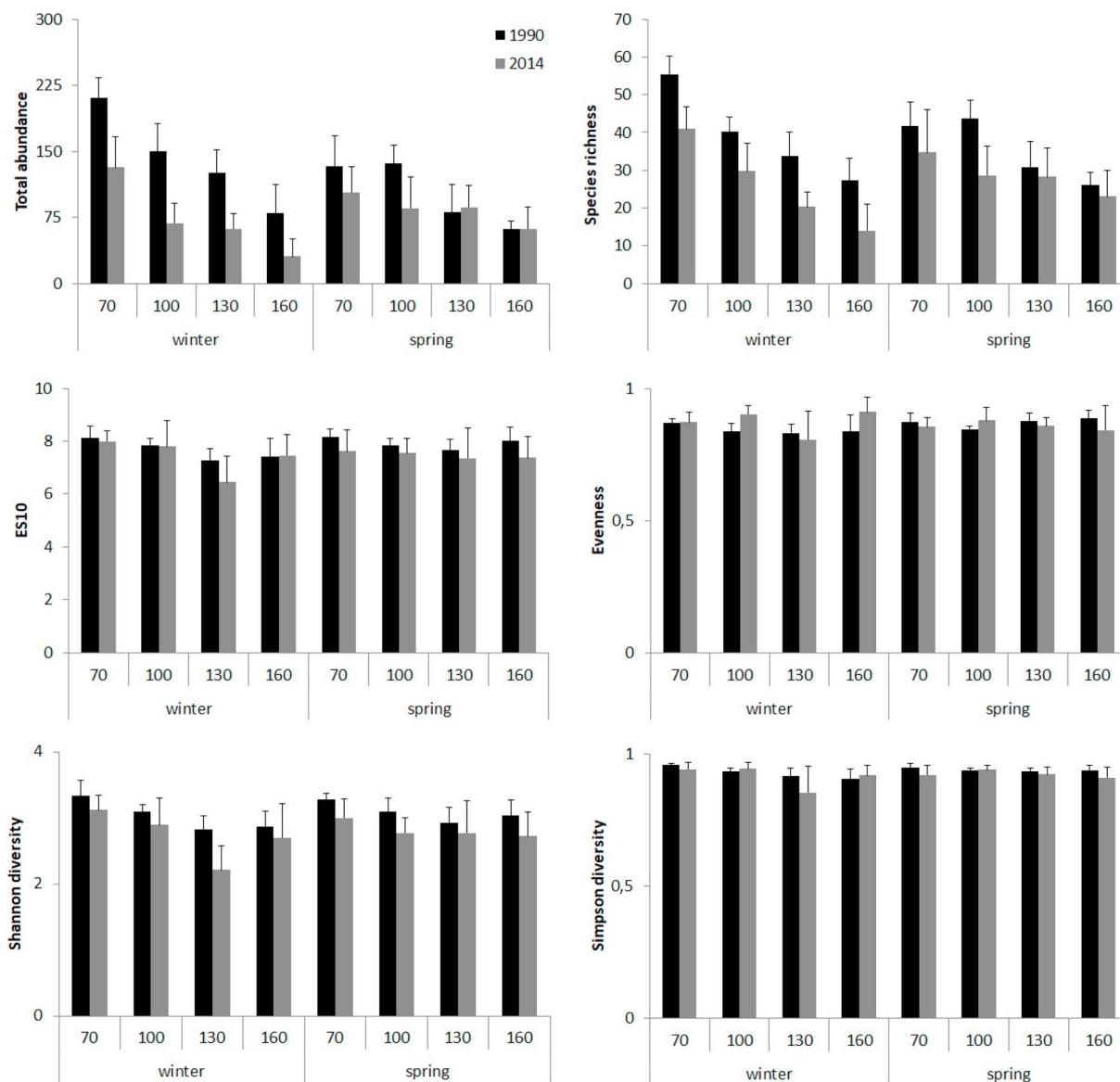


Fig. 2. Temporal change in diversity indices between 1989-90 and 2013-14. Black bars indicate samples collected in 1990 and gray bars in 2014. Error bars indicate standard deviation.

composition (Fig. 3). Among all genera, 30% of them remained in their historical frequency classes, while 24% of the species changed from rare to absent (Table 2). In contrast, 22% of the species showed increasing occurrence changing from absent to rare (Table 2).

Temporal changes in the accumulation curves for the 1989-90 and 2013-14 samplings at both seasons are shown in Fig. 4. Both rarefaction curves and their extrapolation components were higher in 2013-14 than in 1989-90, this difference was higher in winter than in spring (Fig. 4).

Table 1

Three-way ANOVA results for the temporal comparisons of the diversity metrics. Significant differences at $p < 0.05$ indicated with “**”, at $p < 0.01$ indicated with “***” and non-significant indicated with “ns”.

	df	Total Abundance		Species richness		ES10		Evenness		Shannon diversity		Simpson diversity	
		F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.
Corrected Model	15	26.9	**	22.439	**	5.148	**	2.672	**	12.912	**	4.309	**
Intercept	1	1928.2	**	3468.9	**	22327.6	**	44359.2	**	18912.3	**	105817.5	**
Year	1	92.368	**	81.547	**	9.378	**	1.16	ns ^a	39.256	**	7.041	**
Season	1	8.724	**	0.296	ns	1.859	ns ^a	0.534	ns ^a	2.517	**	2.843	ns ^a
Depth	3	63.686	**	65.722	**	14.586	**	2.243	ns ^a	42.206	ns ^a	9.298	**
Year * Season	1	33.445	**	7.571	**	0.252	ns ^a	6.131	ns ^a	1.37	ns ^a	0.031	ns ^a
Year * Depth	3	5.836	**	1.057	ns	2.914	ns ^a	3.518	ns ^a	1.17	ns ^a	2.634	ns ^a
Season * Depth	3	9.153	**	8.056	**	4.412	**	2.985	ns ^a	6.246	**	5.066	**
Year * Season * Depth	3	0.728	ns	2.709	*	1.035	ns ^a	2.643	ns ^a	2.093	ns ^a	2.638	ns

^a At level $\alpha = 0.01$.

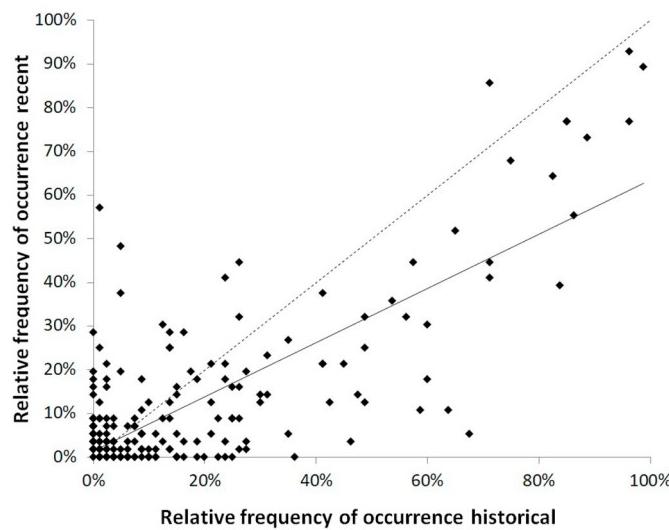


Fig. 3. Temporal changes in the relative frequency of genus occurrence in all samples (historical, 1989–90; recent, 2013–14). Solid line indicates the trend line and dotted line indicates equal occurrences across both datasets.

Table 2

Changes in species classification to occurrence classes in the two sampling periods.

		Recent			
		Absent	Rare	Intermediate	Common
Historical	Absent	0	62 (22.3%)	5 (1.8%)	0
	Rare	67 (24.1%)	40 (14.4%)	11 (4.0%)	1 (0.4%)
	Intermediate	12 (4.3%)	17 (6.1%)	33 (11.9%)	0
	Common	0	1 (0.4%)	10 (3.6%)	11 (4.0%)

3.3. Benthic community composition and species co-occurrence patterns

PERMANOVA detected a significant Year*Season*Depth interaction (Table 3), indicating that differences in benthic community composition varied at least across one factor considered in this study. Since we were interested in the effect of the sampling year on the benthic community, only the *post hoc* comparisons of assemblages between 1989–90 and 2013–14 at the different levels of season and depth were presented

Table 3

Results of PERMANOVAs testing for differences in benthic community across the factors year, season and depth. Each term was tested using 999 random permutations of appropriate units. Significant differences ($p < 0.001$) indicated with “***” and non-significant with “ns”.

Source	df	MS	Pseudo-F	Sig.
Year	1	21818	18.086	***
Season	1	4560.2	3.7802	***
Depth	3	18674	15.48	***
Year*Season	1	3407.6	2.8248	***
Year*Depth	3	4983	4.1307	***
Season*Depth	3	2915	2.4164	***
Year*Season*Depth	3	2613.4	2.1664	***
Residuals	120	1206.3		

Pairwise tests for pairs of levels Year

Depth	Season	t	Sig.
70	Winter	3.2526	***
	Spring	2.5162	***
100	Winter	2.6267	***
	Spring	2.3546	***
130	Winter	2.3362	***
	Spring	1.9538	***
160	Winter	2.4767	***
	Spring	1.0724	ns

(Table 3). This multivariate analysis on abundance data indicated differences in macrofaunal composition between 1989–90 and 2013–14 at all depths and seasons, except for the 160 m station in spring. The nMDS plot reflected these results, too (Fig. 5).

Results of RDA have shown that the first two axes explained approximately 56% of the total variation of benthic macrofaunal composition (Monte Carlo permutation test; pseudo-F = 2.9, $p = 0.002$). The horizontal axis mostly corresponded to a depth gradient with deep stations on the left side and shallow stations on the right side. The vertical axis accounted for 22% of the total community variance and separated the samples taken in 1989–90 from those collected in 2013–14 (Fig. 6). Redox potential and temperature are also two significant explanatory environmental variables associated with this ordination.

The top ten genera that contributed most to dissimilarities between years for each depth station, their abundances and functional characteristics are shown in Table 4. The community dissimilarity between years was almost stable for every depth station. At 70 m, the

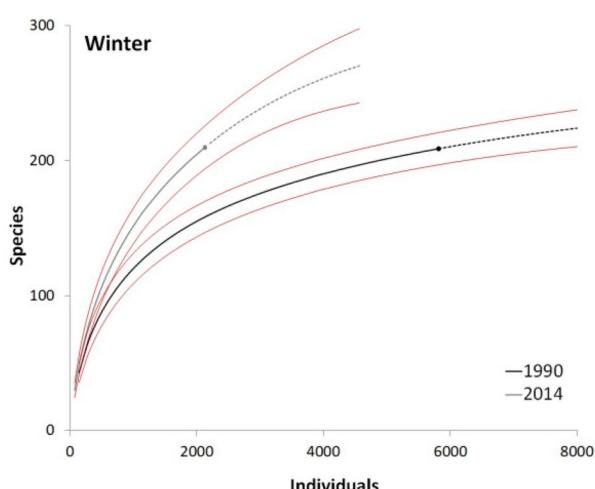
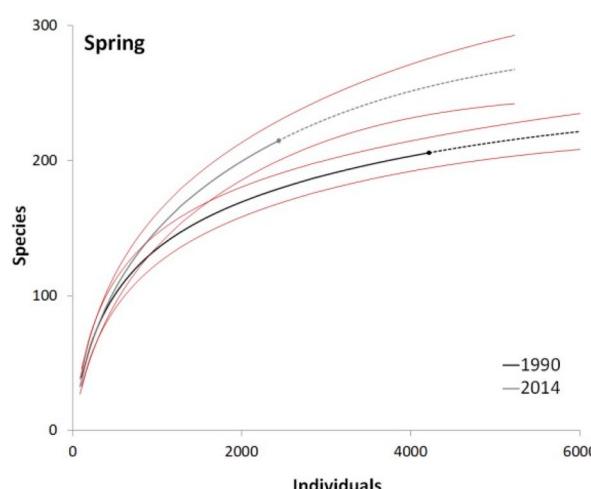


Fig. 4. Sampled-based species accumulation curves for each sampling year. Black line indicates samples collected in 1989–90 and gray line in 2013–14. Circles correspond to reference samples. The parts of the curves to the left of these points correspond to rarefaction curves, whereas the dashed parts of the curves to the right of these points correspond to extrapolation curves. Red curves indicate the standard error. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

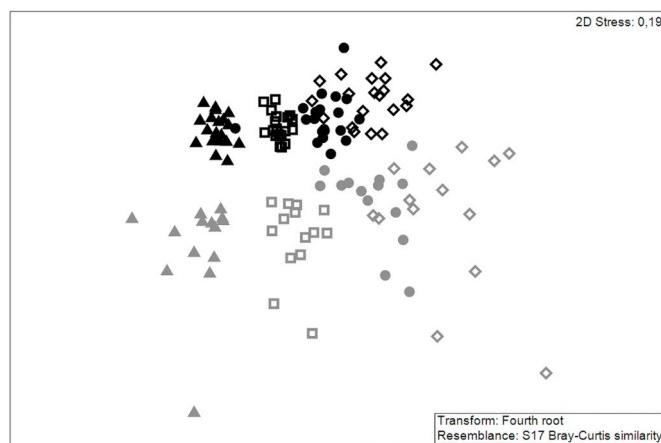


Fig. 5. Non-metric MDS ordination plot for genus abundance data. Triangles indicate samples at 70 m depth; squares indicate samples at 100 m depth; circles indicate samples at 130 m depth and diamonds indicate samples at 160 m depth. Black and gray symbols indicate samples collected in 1989-90 and 2013-14, respectively.

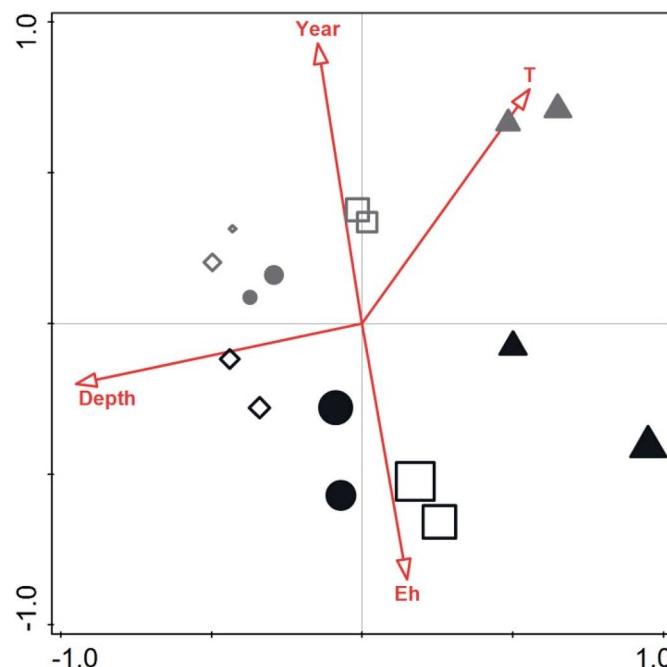


Fig. 6. RDA ordination bi-plot for species abundance data with environmental variables represented as arrows. Triangles indicate samples at 70 m depth; squares indicate samples at 100 m depth; circles indicate samples at 130 m depth and diamonds indicate samples at 160 m depth. Black and gray symbols indicate samples collected in 1989-90 and 2013-14, respectively. Symbol size varies, reflecting the count of species within samples.

dissimilarity between years was mainly due to the decrease in genus abundances (Table 3). It is also worth noting that molluscan genera with low sensitivity values (family ES₅₀; 0.05) showed a decrease in 2013–14, while the polychaetes *Hyalinoecia* showed the highest increase. At 100 m, differences in community composition were attributed to the decline in abundances from 1989 to 90 onwards and again to the increase in *Hyalinoecia*. Similar decreases in genus abundance occurred at 130 m and 160 m depth. The genera with the strongest positive abundance trends were the polychaetes *Chirimia* and the sipunculans *Onchnesoma* at 130 m and 160 m, respectively (Table 3).

The probabilistic co-occurrence analysis showed mostly random genus co-occurrence patterns in 1989–90 (85%) and 2013–14 (86%).

Nevertheless, the results suggested some pairs with positive associations whereas other pairs had negative co-occurrences. Specifically, in 1989–90, the pairs of genera that were positively associated amounted to about 70%, while in 2013–14 the positive associations were 78%. The co-occurrence pattern of the genera which mostly contributed to community composition dissimilarities between 1989–90 and 2013–14 were presented in Table 5, where only positive and negative co-occurrences are shown. *Magelona* and *Hyalinoecia* had the most positive associations, while *Rhodine* and *Ennucula* had the most negative in 1989–90 and 2013–14, respectively (Table 5).

3.4. Benthic ecological quality assessment

The assessment of benthic ecological quality status showed that ecological status fluctuated between the sampling years for all depths as indicated by M-AMBI, BENTIX and BQI-family (Fig. 7). Despite that there were statistically significant differences in the indices for the factors tested (Table 6), their scores were always above the acceptable boundaries indicating good or high ecological status (Fig. 7). The relationships between benthic ecological quality indicators are shown in Fig. 8. M-AMBI and BQI-family were positively correlated ($n = 136$, $r^2 = 0.264$, $p = 0.001$). M-AMBI was also positively correlated to BENTIX ($n = 136$, $r^2 = 0.3$, $p < 0.001$). BQI-family and BENTIX were correlated negatively ($n = 136$, $r^2 = 0.522$, $p < 0.001$).

4. Discussion

Improvement in analytical methods and instruments over time has resulted in an increase in the number of records of benthic invertebrates reported from the Mediterranean Sea (Coll et al., 2010). The present study investigated the changes in diversity and community composition of benthic macrofaunal species from the north coast of Crete based on two datasets (1989–90 and 2013–14). Both datasets, historical and recent, had identical sampling designs and sampling seasons and as a result sampling bias is considered to be minimal in the generated data. In addition, to further achieve data homogeneity between years, statistical analyses were performed at the genus level since identifying organisms to the sufficient taxon minimizes the error of taxonomic identification (Warwick, 1988).

Factors that could potentially induce long-term changes in benthic communities could be divided in three categories: large-scale factors, local factors and stochasticity. Large-scale factors such as climatological shifts or increase in nutrient inputs affecting the pelagic primary production could result in the modification of benthic communities (Katara et al., 2008; Kröncke et al., 2011; Tunberg and Nelson, 1998). Climate change and the increasing trend in nutrient concentration due to atmospheric and terrestrial inputs from anthropogenic activities (Béthoux et al., 1998; Krom et al., 2004) could affect the entire benthic ecosystem in a similar pattern through an increase in primary production and the consequent increase in sedimentation of organic material on the seabed (Clare et al., 2017; Danovaro et al., 2001; Katara et al., 2008; Marbà et al., 2015). This was the case where Kröncke et al. (2011) linked the observed increase in the abundance and diversity of the North Sea macrofaunal communities to the shift in sea surface temperature and primary production. Similar relationships between climate change and macrobenthic community characteristics and diversity have often been recorded around the world (Blanchard, 2015; Dippner and Ikauniece, 2001; Kröncke et al., 2001; Longobardi et al., 2017). On the other hand, local effects such as changes in riverine inputs or discharges of domestic wastes could also modify benthic communities by affecting species recruitment due to physical disturbance and/or organic matter release (Bonifácio et al., 2018; Labrune et al., 2007). In such cases, the pattern of change is expected to be localized and induce greater changes in shallow areas close to the coastal zone. Finally, stochastic events and natural variability could be responsible for long-term differences in benthic ecosystems (Kröncke and Reiss, 2010; Labrune et al., 2007).

Table 4

Average abundance and functional traits of top ten genera contributed most to dissimilarities between years for each depth station.

Genus	Bioturbation	Feeding Group	Sensitivity value (ES _{family})	Av. Abund	2013-14
Depth: 70 m					
Average dissimilarity: 59.72					
<i>Euclymene</i>	UC/DC	deposit feeder	13.94	46.7	0.2
<i>Anapagurus</i>	E	scavenger	12.4	16.3	0.0
<i>Edwardsia</i>	S	predator	14.23	21.4	0.0
<i>Hyalinoecia</i>	S	scavenger	20.11	11.3	141.6
<i>Chondrochelia</i>	S	detritus feeder	10.51	0.0	12.6
<i>Diplocirrus</i>	UC	deposit feeder	16.43	18.1	0
<i>Corbula</i>	S	suspension feeder	4.84	22.2	0.1
<i>Abra</i>	S	deposit feeder	4.82	15.7	0.0
<i>Timoclea</i>	S	suspension feeder	1.91	6.9	0
<i>Chone</i>	S	suspension feeder	10.01	1.0	22.2
Depth: 100 m					
Average dissimilarity: 59.71					
<i>Axinulus</i>	DC	deposit feeder	10.53	79.7	0
<i>Falcidens</i>	S	deposit feeder	14.89	13.6	0
<i>Magelona</i>	S	deposit feeder	17.62	14.6	0.1
<i>Scleotoma</i>	B	predator	13.37	8.1	0.1
<i>Hyalinoecia</i>	S	scavenger	20.11	0	3.9
<i>Chaetozone</i>	S	deposit feeder	7.04	4.1	0.2
<i>Euclymene</i>	UC/DC	deposit feeder	13.94	26.0	1.9
<i>Tachytrypane</i>	B	deposit feeder	7.24	3.9	0.5
<i>Levinsenia</i>	S	deposit feeder	12.64	238.5	54.8
<i>Perioculodes</i>	S	predator	12.14	2.4	0.0
Depth: 130 m					
Average dissimilarity: 58.43					
<i>Axinulus</i>	DC	deposit feeder	10.53	29.4	0.0
<i>Amphiura</i>	B	deposit feeder	2.87	13.1	0.0
<i>Chirimia</i>	UC/DC	deposit feeder	13.94	0.0	8.5
<i>Euclymene</i>	UC/DC	deposit feeder	13.94	17.5	1.2
<i>Tachytrypane</i>	B	deposit feeder	7.24	7.5	1.8
<i>Monticellina</i>	S	deposit feeder	7.04	60.8	9.6
<i>Rhodine</i>	UC	deposit feeder	13.94	26.8	4.3
<i>Aphelochaeta</i>	S	deposit feeder	7.04	46.7	9.6
<i>Prionospio</i>	UC/DC	deposit feeder	4.63	5.0	8.9
<i>Ennucula</i>	S	deposit feeder	11.41	0.0	2.1
Depth: 160 m					
Average dissimilarity: 61.55					
<i>Rhodine</i>	UC	deposit feeder	13.94	40.6	0.8
<i>Tachytrypane</i>	B	deposit feeder	7.24	11.2	0.1
<i>Monticellina</i>	S	deposit feeder	7.04	12.6	0.4
<i>Onchnesoma</i>	S	deposit feeder	13.37	0.0	5.2
<i>Prionospio</i>	UC/DC	deposit feeder	4.63	15.2	3.3
<i>Glycera</i>	B	predator	12.66	6.9	0.5
<i>Axinulus</i>	DC	deposit feeder	10.53	2.1	0.0
<i>Marphysa</i>	B	predator	16.21	2.5	0.2
<i>Ennucula</i>	S	deposit feeder	11.41	0.2	1.5
<i>Aricidea</i>	S	deposit feeder	4.63	3.2	3.7

Bioturbation types: S for surficial modifiers, B for biodiffusors, UC and DC for upward and downward conveyors (Queirós et al., 2013).

However, under these processes, it is anticipated that no significant changes in functional types or directional trends would occur.

The results of the present study indicated the existence of statistically significant differences in benthic communities (diversity and species composition) between 1989-90 and 2013-14. Specifically, there was a decrease in total abundance and average species richness per sampling unit between the two sampling periods. These differences were more important in the shallow stations and, as a result, they could be explained by local factors. Shallow stations are more disposed to resuspension events and consequently are more susceptible to natural disturbance (Labrune et al., 2007), and they are affected also by anthropogenic activities in the coastal zone. In agreement with our results, a negative trend in benthic diversity was recorded in the Gulf of

Lion (western Mediterranean) and attributed to the NAO and the consequent resuspension events as well as to the local effect of riverine inputs affecting species recruitment (Bonifácio et al., 2018; Labrune et al., 2007). In the case of the north coast of Crete, the change in benthic communities could be attributed to the construction of the Heraklion municipality wastewater treatment plant. In such an oligotrophic environment, this decrease in organic material and nutrient inputs has probably led to the reduction in macrofaunal abundance. This, in turn, has affected some of the sample-size dependent diversity indices such as species per grab and Shannon H' and less so for Simpson index which is known to be less sensitive to the sample size (Karakassis et al., 1996).

The recent sampling also showed a higher yield of species for a

Table 5

Changes in genus co-occurrence pairs (positive and negative) for the top ten genera mostly contributed to dissimilarities between years for each depth station. Bold types indicate genera with maximum positive and negative co-occurrences.

	Positive co-occurrences		Negative co-occurrences	
	1989–90	2013–14	1989–90	2013–14
<i>Abra</i>	26	4	5	0
<i>Amphipura</i>	9	26	4	5
<i>Anapagurus</i>	38	0	12	0
<i>Aphelochaeta</i>	17	25	5	6
<i>Aricidea</i>	8	9	2	1
<i>Axinulus</i>	11	0	12	0
<i>Chaetozone</i>	35	28	10	4
<i>Chirimia</i>	0	5	1	1
<i>Chondrochelia</i>	0	22	0	8
<i>Chone</i>	27	24	5	3
<i>Corbula</i>	47	9	13	1
<i>Diplocirrus</i>	40	0	12	0
<i>Edwardisia</i>	18	0	9	0
<i>Ennucula</i>	5	2	15	16
<i>Euclymene</i>	6	7	2	4
<i>Falcidens</i>	29	3	5	0
<i>Glycera</i>	0	15	7	2
<i>Hyalinoecia</i>	39	41	13	7
<i>Levinsenia</i>	2	2	6	1
<i>Magelona</i>	51	38	13	3
<i>Marpysa</i>	6	2	11	3
<i>Monticellina</i>	4	18	3	1
<i>Onchnesoma</i>	41	0	14	3
<i>Perioculodes</i>	39	0	4	0
<i>Prionospio</i>	9	6	2	1
<i>Rhodine</i>	11	10	40	1
<i>Scoletoma</i>	3	8	2	1
<i>Tachytrypane</i>	7	10	29	6
<i>Timoclea</i>	37	0	8	1

certain sampling effort and an increase, although not statistically significant, in the species evenness. This difference may be due to the dominance of a few species with high abundances in the historical samples (Obst et al., 2017). Species occurrences and multivariate patterns of benthic communities were indicative of temporal changes in species composition. This temporal variation was associated with sediment temperature and redox potential. High temperatures seemed to determine the community composition in shallow stations, particularly in 2014, and the decrease in redox potential appeared to be responsible for the differences between the two sampling periods. Natural variability, which was responsible for the fluctuation in sediment temperature and redox potential, is an important factor in controlling benthic variability (Tunberg and Nelson, 1998). Temperature and dissolved oxygen act as limiting factors in structuring macrofaunal communities, by regulating and setting limits for recruitment and mortality (Hale et al., 2011; Hiscock et al., 2004; Pollack et al., 2011; Valentine and Jablonski, 2015).

The temporal variation in the shallow stations was due to the local extinction or population decline of certain species, mainly deposit, suspension feeders or predators and the dominance of the polychaete *Hyalinoecia* in 2013–14. Representatives of this large genus group are described as scavengers as well as surface deposit feeders (Jumars et al., 2015) inhabiting nutrient-poor soft bottoms. Their dietary flexibility, particularly in the case of low food availability (Cosentino and Giacobbe, 2008) supports the hypothesis of a reduction in nutrient input in the study area after the establishment of the wastewater treatment plant. At the deeper stations, the community varied quantitatively rather than qualitatively. Nevertheless, the successful colonization of these depths by the sipunculans *Onchnesoma* sp. may be attributed to the competitive advantages derived from their ability to obtain food deeper in the sediment and to adapt to environments characterized by temporary sedimentation (Karakassis and Eleftheriou,

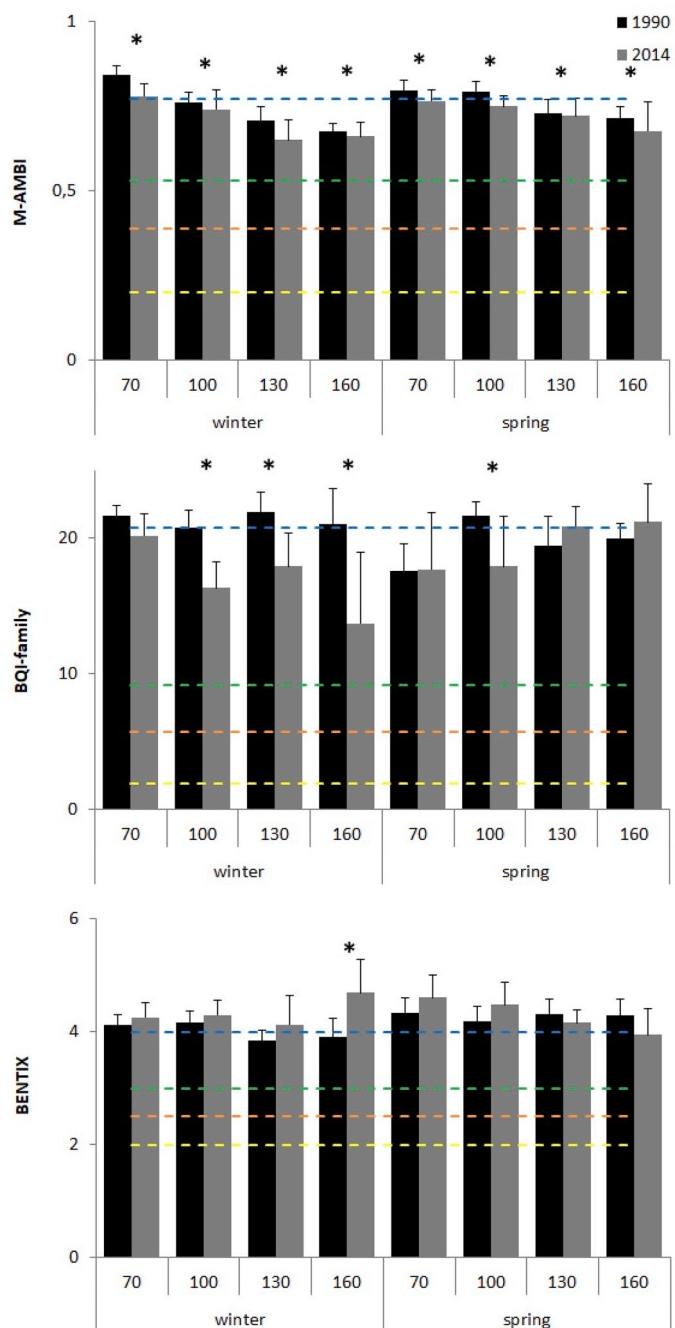


Fig. 7. Temporal change of ecological indicators (M-AMBI, BQI-family, BENTIX) during 1990–2014. Black bars indicate samples collected in 1990 and gray bars in 2014. Error bars indicate standard deviation. Significant differences ($p < 0.05$) between 1989-90 and 2013-14 were indicated by **. Horizontal dashed lines indicate boundaries of the different Ecological Status used in the context of the Water Framework Directive for each indicator (Starting from the bottom Bad, Poor, Moderate, Good, High).

1997), indicating once again the low food availability at the ecosystem.

In addition to the local effects, the comparison of species occurrence in the historical and the recent inventory as well as the lack of pattern in genus co-occurrence suggested that there was also an important stochastic element in the benthic community change in the study area. This stochasticity could also explain the seasonal effect on the community composition and total abundance comparisons between 1989–90 and 2013–14. Moreover, the effect of natural variability on benthic communities is supported by the long-term comparison of three biotic indices which indicated a lack of change in the ecological quality status

Table 6

Three-way ANOVA results for the temporal comparisons of the benthic ecological quality indices. Significant differences at $p < 0.05$ indicated with “*”, at $p < 0.01$ indicated with “**” and non-significant indicated with “ns”.

	M-AMBI		BQI-family		BENTIX		
	df	F	Sig.	F	Sig.	F	Sig.
Corrected Model	15	14.449	**	47.344	**	4.388	**
Intercept	1	40857.7	**	8671.1	**	24778.3	**
Year	1	21.925	**	30.032	**	12.566	**
Season	1	4.989	*	0.711	ns	6.266	*
Depth	3	53.138	**	1.195	ns	3.019	*
Year * Season	1	0.257	ns	23.855	**	7.150	**
Year * Depth	3	0.391	ns	3.518	*	0.751	ns
Season * Depth	3	4.717	**	10.722	**	2.986	*
Year * Season * Depth	3	1.597	ns	4.751	**	6.601	**

between the two sampling periods. Changes in abundance levels or species numbers are reflected in univariate diversity indices not taking into account the ecological role or the life strategies of the species involved. In contrast, indices such as M-AMBI and BQI-family are mainly affected by the relative abundance of tolerant/sensitive taxa in the sample, thereby remaining unaffected by changes in the total number of species as long as these changes do not involve major changes in the life strategies of the species within the community. In this context, they are less sensitive to seasonal variability of benthic communities (Karakassis et al., 2013; Kröncke et al., 2011; Reiss and Kröncke, 2005; Romero-Ramirez et al., 2016). So long as the ecological quality in the study area has not changed positively or negatively over time, the recorded changes in benthic composition between 1990 and 2014 did not indicate an increase either of opportunistic species or K-strategists but rather a substitution of species with similar functional/ecological roles.

Comparison of the values of three ecological indicators of samples taken in 1989-90 and 2013-14 provides an almost identical conclusion regarding the ecological quality in the north coast of Crete. This is quite common and many studies have addressed an agreement among benthic quality indicators (Kennedy et al., 2011; Kröncke and Reiss, 2010; Romero-Ramirez et al., 2016). Nevertheless, in some samples the three indices showed different outcomes. Some authors (Kennedy et al., 2011; Kröncke and Reiss, 2010) have suggested that this discordance originates from the different responses of these indicators to natural variability, freshwater input and storm events as well as how resilient they are.

Despite the changes in benthic community composition and the few discrepancies between the biotic indices, ecological status remained above the threshold values for good ecological status in the north coast of Crete both in 1989-90 and 2013-14. All indicators are quite effective in absorbing the natural variability in benthic communities and highlighting anthropogenic impacts (Borja et al., 2007; de Paz et al., 2008; Kröncke et al., 2011; Simboula et al., 2007).

5. Conclusions

After a quarter of a century the benthic ecosystem in the north coast of Crete changed. Benthic communities showed a lower abundance during the recent sampling period and a rather small but statistically significant decrease in diversity metrics. Species accumulation curves revealed that for a certain number of individuals more species were recorded in 2013-14 indicating an even distribution of species abundances in the recent communities. Changes in the community composition and species co-occurrences seemed to have been driven by a combination of local anthropogenic factors and stochasticity due to meteorological parameters rather than by macro-ecological factors. Despite these changes, benthic ecosystem has maintained its good ecological status indicating that benthic ecological indicators are quite conservative and could be used for long-term comparisons.

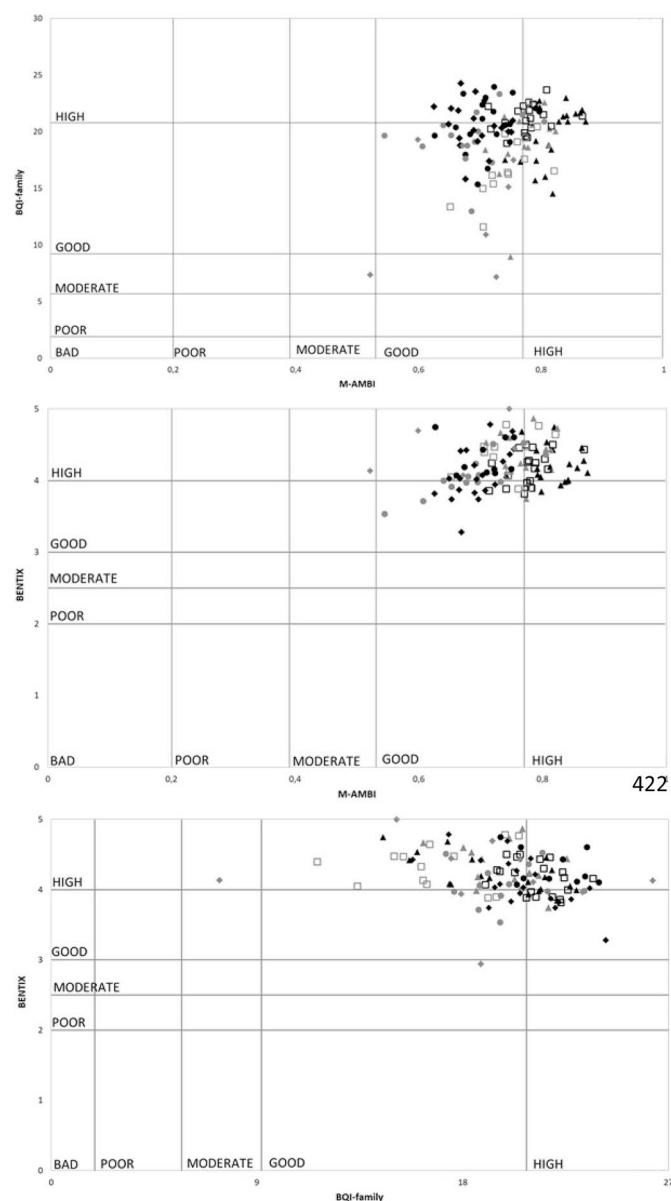


Fig. 8. Comparison of a) BQI-family and M-AMBI, b) BQI-family and BENTIX, c) M-AMBI and BENTIX. Horizontal and vertical lines indicate the boundaries of the different Ecological Status used in the context of the Water Framework Directive. Triangles indicate samples at 70 m depth; squares indicate samples at 100 m depth; circles indicate samples at 130 m depth and diamonds indicate samples at 160 m depth. Black symbols indicate the samples collected in 1989-90 and gray symbols indicate the samples taken in 2013-14.

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Author contributions

I.T. and I.K. planned the hypothesis and designed the sampling, A.E., C.J.S., K.N.P. and W.P. provided the historical data, I.T., M.L.M and A.G. identified macrobenthic species. I.T. collected the raw data, performed the analyses and wrote the first version of the manuscript. All co-authors contributed to revisions.

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Appendix A. Supplementary data

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